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INHALATION EXPERIMENTS ON INFLUENZA AND PNEUMONIA, AND ON THE IMPORTANCE OF SPRAY-BORNE BACTERIA IN RESPIRATORY INFECTIONS

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While the influenza virus appeared to be of reduced virulence during the outbreak of February and March, 1920, the occasional occurrence of a family outbreak, or of rapid death due to pulmonary edema, seemed to indicate that it was identical with that present in the outbreak of 1919. We feel particularly sure that we were dealing with cases of influenza in the family S., because the mother, father and eight children all came into the hospital at once. Most of these ten patients had bronchopneumonia and one purulent pleuritis. Cases R. and S., B. and B. and T. were of a milder type, although B. and T. had secondary bronchopneumonia. These cases were chosen because most of them had been ill for only a day or two at the time the material was collected.

Thirty-eight cases, including those just cited, were examined bacteriologically. All aerobic, partial tension and anaerobic blood cultures were negative. For the throat and sputum cultures we used + 0.5 agar containing 5% of rabbit blood. Incubation was at 37 C. under aerobic, partial tension and anaerobic conditions. B. influenzae was isolated from 12 cases. Six of the 12 were from the family S. who all became ill at once. From the father of this family we failed to isolate B. influenzae, and he was ill for months with streptococcus empyema. In 6 cases B. influenzae was the predominating organism and in 4 it was present in pure culture.

When B. influenzae was grown at partial tension it retained its minute bipolar form and showed less tendency to involution than when grown aerobically. All the strains were strictly hemoglobinophilic while the Koch-Weeks bacillus was isolated on aerobic, partial tension and anaerobic slants of ascites agar, and could be subcultured

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on this medium. All strains failed to produce indol in hemoglobin broth when the sulphuric acid-sodium nitrite test was applied. However, the growth in our broth was scanty. Most of the associated bacteria belonged to the pneumococcus, hemolytic streptococcus and staphylococcus groups. No colonies that might have represented the B. enteritidis (type M 5), which appeared in the sprayed animals, were noted, and it is not likely that they were overlooked, for M 5 colonies on blood-agar plates at partial tension are a vivid green—as are those of typhoid, paratyphoid B. and B. enteritidis.

Antigens from all strains tested were prepared at one time and kept under the same conditions. Cultures were washed and suspended in 0.9% salt solution, killed at 65 C. for 30 minutes, freed of clumps and preserved with 0.5% phenol.

Agglutinating serum for B. influenzae and the Koch-Weeks bacillus were prepared by inoculating rabbits with living cultures. In the case of M 5 a dead antigen was necessary. The serums had a rather low titer, about 1 : 800.

TABLE 1

AGGLUTINATION AND ABSORPTION REACTIONS

Designation of Strains	Mother Antiserum		Koch-Weeks Antiserum	
	Simple Agglutination	Homologous Agglutinins Absorbed	Simple Agglutination	Homologous Agglutinins Absorbed
1. Mother	+	+	—	
2. B.	+	+	—	
3. H.	+	P	+	P
4. S.	+	+	—	
5. M.	+	+	—	
6. S.	—	..	—	
7. C.	+	P	—	
8. A.	—	..	—	
9. Sum.	—	..	—	
10. K.	—	..	—	
11. Leo	—	..	—	
12. Koch-Weeks	—	..	+	+

+ means positive agglutination.
— means no agglutination.
P means homologous agglutinins.
P means homologous agglutinins only partially absorbed. When simple agglutination tests were negative absorption tests were not made.

As shown in table 1, four of the strains from the family S., mother, B., S. and M., and strain C from a healthy individual, are probably identical. One other of the family strains, H., is somewhat related to this group but is about as closely related to the Koch-Weeks bacillus. This agrees with the work of others in showing that there are distinct serologic groups among the influenza bacilli and that some strains are closely related to the Koch-Weeks bacillus.

Blood serum from some of the recovered members of family S., who had harbored B. influenzae, contained no agglutinins for this organism.

ATTEMPTS TO TRANSMIT INFLUENZA TO WHITE MICE AND RATS, GUINEA-PIGS AND RABBITS BY MEANS OF SPRAYED SPUTUM

Sputum, or, when this could not be obtained, material swabbed from the tonsillar area, but generally both, were thoroughly shaken with 0.9% salt solution and sprayed by means of a Vilbiss atomizer within half an hour after collection. The animals were placed in a metal box about 12 x 10 x 6 inches, provided with a glass window and air outlets. The spraying was continued until the chamber was filled with vapor. This was repeated at intervals until 15-20 c c had been sprayed. After from 30-60 minutes the animals were removed from the cage and each series kept in separate cages. These were scalded with hot water before they were used for any given series. The sawdust bedding was not sterilized. Precautions were taken to sterilize the drinking pans in the case of all mouse experiments. The animals were fed on cracked maize and vegetable waste from the hospital kitchen.

It might be noted here that one of us in Cincinnati inoculated sterile milk with influenza sputum (1919) and incubated it at 37, 24 and 15 degrees for from 1-14 days, and fed it to white rats and white mice. Of about 40 animals so fed only 2 mice died, one with pneumococcus septicemia, and one with pneumonia and serous pleuritis due to 4 different bacteria. None of these bacteria, singly or combined, produced infection when fed to other mice in milk or broth cultures.

Table 2 gives the data on these experiments.

Family S., Sputum Spray: Four of 5 mice died infected with a strain of B. enteritidis (type M 5); two of these died of a primary pneumonia due to M 5. By the term primary pneumonia we mean pneumonia without marked involvement of the liver and spleen, which invariably occurs in a general infection, e. g., after feeding.

Of 2 guinea-pigs, 1 died of primary pneumonia due to M 5. One of 2 mice and 1 of 2 guinea-pigs sprayed with extracts of the spleen, liver and lungs of this animal died of general infection with M 5.

Two of 4 rats died of primary pneumonia and the lung cultures yielded no growth, nor were subinoculations of organ extracts fatal to rats.

Partial tension rabbit blood-agar plate cultures, from the sputum used for spraying the animals, were sprayed after 24 hours' growth at 37 degrees. Of 6 mice, 2 guinea-pigs and 1 rabbit, only 3 animals died—2 mice the 31st and

TABLE 2

INFLUENZA-SPUTUM SPRAYING EXPERIMENT

Date	Material Used to Infect Source	Method of Exposure	Number and Species of Animals Exposed	Number of Animals Fatally Infected	Number of Infected Animals with Pneumonia	Minimum and Maximum Duration of Illness, Days	Percentage of Fatalities	Remarks
2/ 3/20	Sputum S	Spray	5 mice	4 (1 killed)	2 (M1,M2)	19–73	80	All like M5
2/ 3/20	Sputum S	Spray	4 white rats	2	2 (W1,W2)	13	50	Sterile
2/ 3/20	Sputum S	Spray	2 guinea-pigs	2	1 (G.-P. 3)	8–80	100	Like M5
2/22/20	Lung emulsion M1	Intraperitoneal	2 mice	2	0	6–8	100	General infection like M5
2/23/20	Mixed culture M1 plate	Spray	2 mice	2	2 (M5,M6)	8–12	100	M6 like M5
3/ 3/20	Lung emulsion M5	Spray	2 mice	1	1	12	50	Like M5
2/25/20	Organ emulsion M2	Spray	2 mice	0	0	..	0	
2/16/20	Organ emulsion W1, W2	Intraperitoneal	2 white rats	0	0	..	0	
4/23/20	Organ emulsion G.-P. 3	Spray	2 mice	1	0	40	50	
3/23/20	Organ emulsion G.-P. 3	Spray	2 guinea-pigs	1	0	30	50	General infection like M5
2/ 4/20	Sputum cultures case S	Spray	6 mice	2	2	31–45	33	No growth obtained from any tissue
2/ 4/20	Sputum cultures case S	Spray	2 guinea-pigs	0	0	..	0	Killed and found normal 5/2/20
2/ 4/20	Sputum cultures case S	Spray	2 white rats	0	0	..	0	
2/ 4/20	Sputum cultures case S	Spray	1 rabbit	1	1 (R1)	8	100	G + coccus present, lung, large numbers
2/16/20	Culture R1	Intravenously, intraperitoneal	2 rabbits	2	2	34–40	100	Infecting agent not R1
3/ 2/20	Sputum R and S	Spray	2 mice	2	1	33–45	100	Like M5
2/ 2/20	Sputum R and S	Spray	1 guinea-pig	1	1	26	100	Like M5
3/ 3/20	Sputum B and B	Spray	8 mice	4	1	30–38	50	Like M5, one sterile
4/ 4/20	Lung emulsion B and B 30-day mouse	Intraperitoneal	2 mice	1	1	50	50	Like M5
4/ 7/20	Lung emulsion B and B 38 day mouse	Intraperitoneal	1 mouse	1	0	3	100	Like M5
3/ 5/20	Sputum B and B	Spray	6 mice	1	0	52	...	Like M5 from lung
3/ 5/20	Sputum T	Spray	6 mice	2	0	72–84	33	Like M5

45th day were sterile bacteriologically, and 1 rabbit of primary pneumonia, apparently due to a gram-positive coccus, which, however, did not reappear in the 2 subinoculated rabbits.

R. and S. Sputum Spray.—The 2 mice sprayed died of infection with M 5, one of primary pneumonia. The guinea-pig died of primary pneumonia due to M 5.

B. and B. Sputum of 3/3.—Of 8 mice, 4 died, but only 1 of these had pneumonia due to M 5. Two had general infection due to M 5.

B. and B. Sputum of 3/5.—Of 6 mice sprayed 1 died on the 52nd day of general infection with M 5. The rest which were killed 3 months later, were found to be normal and did not harbor M 5.

Sputum T.—Of 6 mice sprayed, 2 died on the 72nd and 84th days thereafter; 1 had a general infection with M 5. The remaining 4 were killed 3 months later and found to be normal.

Summary.—The animals sprayed with influenza sputum comprised 33 white mice, of which 4 died of primary pneumonia and 9 of a general infection with M 5. Of this same lot of mice, 6 were sprayed with culture material and of these 2, which died, were sterile on bacteriologic examination; 2 were killed and cultured 2 months later and found to be uninfected; and 2 were used for another experiment in which they survived for a month.

Of 5 guinea-pigs, 2 died of primary pneumonia; the lungs of these animals contained numerous B. enteritidis which were culturally and serologically identical (agglutination and absorption) with M 5.

Of 6 white rats similarly exposed, 2 died of a primary pneumonia due to an unrecognized cause and not transmissible to rats by intraperitoneal inoculation.

The work of Krumwiede, Valentine and Kohn[1] shows that these animals may develop spontaneous infection with members of the paratyphoid-enteritidis group. We did not encounter a single death among our unused stock due to such bacteria, nor were we able to isolate such bacteria from the intestinal tract, liver, spleen and lungs of 6 normal mice. However, the experiments detailed in table 7 show that a certain number of mice, which are intoxicated by killed cultures of M 5 or by the sterile Berkefeld filtrate of broth cultures, develop a secondary infection with M 5. In such endogenous infections, following intoxication, pneumonia occurred only twice in 40 animals.

Furthermore, of 29 mice sprayed with a virulent culture of the pneumococcus, only 1 died of infection with M 5, and this mouse had received a previous dose of M 5 toxin. On the other hand, as shown in table 5, mice exposed to sprayed cultures of M 5 almost invariably died of a primary pneumonia.

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[1] Jour. Med. Res., 1919, 39, p. 449.

In the light of these data one is tempted to believe that the animals developing infection with M 5 were injured in some way by something in the influenza sputum. Nevertheless, the possibility of purely spontaneous infection exists and the question can only be settled by further work with more adequate controls, i. e., an equal number of animals from each lot used for an experiment should have been kept under identical conditions as controls.

INOCULATION OF OTHER ANIMALS

From one of the typical cases, "R," 20 c c of blood was obtained. This was used to inoculate a series of animals not generally used in laboratory experiments with the hope that a susceptible animal might be encountered. These were a pig, 8 weeks old, weighing about 100 pounds, a ferret, an opossum, a salamander, and a black headed nun. None showed any abnormal symptoms during 3 months' observation.

THE CULTURAL AND AGGLUTINATIVE RELATIONSHIP OF M 5 (TABLES 3 AND 4)

Since all the enteritidis-like organisms isolated from the mice and guinea-pigs exposed to sputum sprays corresponded in their agglutination, absorption and cultural characteristics, we used M 5 alone for the comparative study. Unfortunately only two antiserums, M 5, with a titer of 1:800, and paratyphoid B., with a titer of 1:10,000, were available. Table 3 shows that M 5 is entirely distinct from paratyphoid B., but that it is indistinguishable by this test alone from Danysz virus and from B. enteritidis. However, the cultural results (table 4) show that Danysz virus agrees with paratyphoid B. in its failure to ferment xylose, while M 5 agrees with B. enteritidis in the fermentation of this substance. This divergence was brought out by Krumweide et al.[2] We have been helped also in this study by reference to the work of Jordan[3] and preceding articles cited here and that of Winslow, Kligler and Rothberg.[4] The Danysz virus and paratyphoid B. were from the U. S. Hygienic Laboratory and the B. enteritidis was of the Gaertner type and came from Prof. E. O. Jordan in 1901.

Five cultures of B. enteritidis-like organisms isolated from the stools of influenza patients by Sherwood, Downs, and McNaught[5] were sent by Dr. Sherwood. None of these agglutinated with M 5 antiserum.

EXPERIMENTS SHOWING THAT BROTH CULTURES OF M 5 CONTAIN A SOLUBLE TOXIN WITH WHICH AN ANTITOXIN MAY BE PRODUCED

Plain, maltose and dextrose beef infusion broths were tried. It was found that 0.1% dextrose broth (+ 0.5) yielded the most potent toxin. After incubation at 37 C. for 4-5 days the culture was filtered through a Berkefeld N. The filtrate would kill mice in 12-18 hours when 0.05-0.1 c c was injected intraperitoneally. Seventy mice were used in establishing the nature and potency of this toxin. Mice dying of intoxication showed marked injection of the subcutis and congestion of the lungs. Often the pulmonary congestion bordered on consolidation. The lungs often showed numerous capillary hemorrhages. Other organs and tissues appeared normal to the eye.

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[2] Jour. Med. Res., 1919, 39, p. 449.

[3] Jour. Infect. Dis.. 1920, 26, p. 427.

[4] Jour. Bacteriol., 1919, 4, p. 429.

[5] Jour. Infect. Dis., 1920, 26, p. 16.

We found that about 2 c c was the M L D, on intravenous inoculation, for a rabbit weighing 1,800 gm. By inoculation with sublethal doses and gradually increasing the amount at 2-day intervals, over a period of 3 weeks, a rabbit could tolerate 5 c c of a freshly prepared toxin. Eight days after the last dose of toxin the serum of this rabbit would protect mice when mixed with 2 M L D of the toxin and at once inoculated intraperitoneally.

TABLE 3

Simple Agglutination of Certain Strains by B. paratyph. B. and M 5 Antiserum and the Absorption of Homologous Agglutinins from the Same

Designation of Strains	Para B. Antiserum		M5 Antiserum	
	Simple Agglutination	Homologous Agglutinins Absorbed	Simple Agglutination	Homologous Agglutinins Absorbed
M-5	—		+	+
Para B	+	+	+	—
Enteritidis	—		+	+
Danysz virus	—		+	+

TABLE 4

The Morphologic and Cultural Characteristics of M 5 and of Representative Organisms Selected from the Same Group

	Gram Stain	Motility	Litmus Milk		Indol	H_2S Produced	Gelatin Liquified	Voges Proskauer	Endo's Acid	Green Colonies Partial Tension Blood Agar Plates	Dextrose	Maltose	Galactose	Saccharose	Levulose	Lactose	Mannite	Arabinose	Dulcite	Erythrite	Isodulcite	Mannose	Raffinose	Salacin	Xylose
			Initial Acid	Final Strongly Alkaline																					
M5	—	+	+	+	—	+	—	—	—	+	ag*	ag	ag	—	ag	—	ag	ag	ag	—	ag	ag	—	—	ag
Danysz virus	—	+	+	+	—	+	—	—	—	—	ag	ag	ag	—	ag	—	ag	ag	ag	—	ag	ag	—	—	—
B. Para. B.	—	+	+	+	—	+	—	—	—	+	ag	ag	ag	—	ag	—	ag	ag	ag	—	ag	ag	—	—	—
Enteritidis	—	+	+	+	—	+	—	—	—	+	ag	ag	ag	—	ag	—	ag	ag	ag	—	ag	ag	—	—	ag

ag = acid and gas.

In the experiments summarized in table 7 the vaccine was prepared by suspending an agar culture in 0.9% salt solution, heating at 65 C. for 60 minutes and preserving with 0.5% carbolic acid. The density was somewhat greater than that of a 24-hour broth culture of B. typhosus. The dosage was approximately 2 minims for each inoculation. Several tests of the vaccine before and after its use showed that the bacilli were dead.

TABLE 5

EXPERIMENTS SHOWING THAT WHEN ANIMALS ARE EXPOSED TO THE SPRAY OF M 5 CULTURES A LARGE PERCENTAGE DELEVELOP PNEUMONIA, AND THAT SIMILAR RESULTS FOLLOW THE SPRAY OF DANYSZ VIRUS; WHILE A SPRAY OF B. INFLUENZAE ISOLATED 48 HOURS PREVIOUSLY FROM AN INFLUENZA LUNG WAS WITHOUT EFFECT

Date	Animals	Number of Deaths	Percentage of Dead Animals with Primary Pneumonia	Duration of Illness in Days	Remarks
3/12/20	4 mice	4	100	4-20	
3/17/20	10 mice	8	100	4-15	
3/30/20	4 mice	4	75	1-10	Sublethal dose M5 toxin given few hours before spray. One toxin death
3/25/20	5 mice	4	100	4-12	
4/ 8/20	1 mouse	1	0	16	
5/11/20	2 guinea-pigs	2	100	11-17	
5/ 5/20	6 mice	5	100	12-20	Danysz virus spray
	4 mice	0	...	.....	B. influenzae

TABLE 6

EXPERIMENTS SHOWING FAILURE TO IMMUNIZE AGAINST M 5 CULTURE SPRAY BY PREVIOUS INOCULATIONS OF SUBLETHAL DOSES OF M 5 SOLUBLE TOXIN

Date	Number of Mice	Number of Deaths	Duration of Illness in Days	Remarks
4/6/20	4	3	11-18	One dose soluble toxin 6 days before spray.
4/6/20	5	4	12-44	Four doses soluble toxin at 3 day intervals. Last dose 5 days before exposure to spray
4/6/20	4	1	14	Controls without toxin

TABLE 7

EXPERIMENTS SHOWING FAILURE TO IMMUNIZE AGAINST M 5 SPRAY BY PREVIOUS SUBCUTANEOUS INOCULATION WITH A KILLED CULTURE

Date	Number of Mice	Number of Deaths	Percentage of Dead Animals with Primary Pneumonia	Duration of Illness in Days	Remarks
5/7/20	4	4	100	12-23	One dose 10 days before exposure to spray
5/7/20	4	3	33*	1*- 8	Two doses at 4 day interval. Last dose 10 days before spray
5/7/20	4	3	66*	8-14	Three doses at 4 day interval. Last dose 10 days before spray
5/7/20	5	5	80	6-14	Controls not vaccinated

* Two mice died of general infection with M 5 before the time for exposure to the spray.

EXPERIMENTS SHOWING THAT PRIMARY PNEUMONIA IS NOT PRODUCED IN MICE WHEN M 5 IS INOCULATED BY OTHER MEANS THAN SPRAY

When the portal of entry was through the conjunctiva, the buccal and gastro-intestinal mucosa, the subcutis or peritoneal cavity, general infection followed. In two cases there was a secondary pneumonia. In such cases the liver and spleen were greatly enlarged and full of whitish necrotic or proliferative areas. These organic lesions are not present in mice dying of inhalation pneumonia. Placing the culture on the nasal mucosa was without effect.

The mice fed M 5 were kept without food and water for from 1-2 days and then each one was watched while it lapped up the drops of a 24-hour broth culture. Those fed heavily ate bread soaked with the broth culture.

TABLE 8

EXPERIMENTS SHOWING THAT PRIMARY PNEUMONIA IS NOT PRODUCED IN MICE WHEN M 5 IS INOCULATED BY OTHER MEANS THAN SPRAY

Date	Method of Inoculation	Number of Mice	Number of Deaths	Percentage with Pneumonia	Duration of Illness in Days	Remarks
3/18/20	Fed heavily	4	3	0	6-14	General infection
3/31/20	Intraperitoneal	4	4	0	2-4	General infection
4/20/20	Fed 1 drop	4	1	0	8	General infection
4/20/20	Fed 5 drops	4	3	0	10-18	General infection
4/20/20	Fed heavily	2	2	50	8-9	General infection. one with secondary pneumonia
4/20/20	Fed heavily	2	2	0	12-17	General infection
5/11/20	Subcutaneous	3	3	0	2-5	General infection
5/11/20	Ocular conjunctiva	3	2	50	9-19	General infection, one with secondary pneumonia
5/11/20	Anterior nares	4	0			

EXPERIMENTS SHOWING THAT SPRAYED BACTERIA ARE ACTUALLY INHALED INTO THE DEEPEST PARTS OF THE LUNGS

(a) Four mice and one guinea-pig were sprayed with a broth culture of M 5, and chloroformed within 30 minutes from the beginning of the experiment. They were wet with alcohol and immersed in 1:1,000 bichlorid of mercury for 5 minutes. Then they were dissected with aseptic precautions and from each animal 4-6 small pieces of the lungs (1-3 mm. in diameter) were snipped off with sterile scissors and planted in broth. In every instance all the pieces yielded growth of M 5 within 24 hours. Many of the pieces of lung represented the extreme distal portions of the anterior and posterior lobes. Five normal mice controls were treated in the same way. All cultures from these remained sterile during 72 hours' observation.

(b) Experiment (a) was duplicated except that a very virulent pneumococcus (type 1) was used and the tissue was planted in glucose rabbit blood broth. The 4 mice were killed and cultured as in experiment (a) at 2, 4, 8 and 18 hour intervals after the spray. In every instance the cultures showed that pneumococci were present in the deepest parts of the lungs. Cultures from one control mouse yielded no growth in the same medium.

ATTEMPTS TO PRODUCE PNEUMONIA IN MICE BY SPRAYING PNEUMOCOCCI

Having shown that sprayed bacteria reach the deepest alveoli, or capillary bronchi, of the lungs and that pneumococci planted in this way survive in the lungs of mice for at least 18 hours, the maximum period tested, we made the following experiments with a type 1 pneumococcus. This culture had been kept highly virulent for mice at the U. S. Hygienic Laboratory.

Four mice were sprayed with the growth from 4 blood agar slants suspended in broth. They were exposed for 30 minutes. Two died of primary lobar pneumonia in 14 days. No bacteria could be found in the purulent exudate and all cultures remained sterile. The remaining 2 were killed 6 weeks later. They appeared normal and cultures from the lungs remained sterile.

Ten mice were kept at 8 C. for 4 hours and then sprayed. They felt warm on removal from the icebox. Two were killed and cultured shortly after spraying. Pneumococci grew out of all pieces of their lungs, including the most distal portions. At the end of 4 weeks the remaining 8 mice were chloroformed and cultured with negative results.

Since M 5 soluble toxin injures the lungs of mice, 6 mice which had survived sublethal doses of this toxin given 10 days before were sprayed. They were killed 6 weeks later and found normal. Nor did they harbor pneumococci.

Four mice were given sublethal doses of M 5 toxin and sprayed with pneumococci at once. Six weeks later one of these mice died of pneumonia caused by M 5; no pneumococci could be found. The remaining 3 were killed and cultured 8 weeks later. They were normal and the cultures remained sterile.

Three mice were sprayed with the pneumococcus and then in an attempt to give them an acidosis they were kept under ether for one hour. One of these was further chilled in ice water for 10 minutes. They survived and yielded no growths 4 weeks later.

Two mice were sprayed with a broth suspension of bloody sputum from a case of pneumococcus lobar pneumonia. They remained well during 6 weeks' observation.

Since none of the 29 mice became infected after inhaling virulent pneumococci into their lungs, one may conclude that some predisposing factor must precede or accompany such an implantation of bacteria. While we owe the whole idea of droplet infection to Flügge and his pupils and confess that we have relied on the review of their work by Goetschlich,[6] we are not aware of the fact if these workers demonstrated that bacteria are to be recovered from the deepest portions of the lungs of sprayed animals. Our attention was drawn to this by Rogers,[7] who showed that tubercle bacilli could be recovered from the lungs of guinea-pigs immediately after spraying them with tuberculous sputum, and that such protected and sprayed animals develop true primary pulmonary tuberculosis.

We are familiar with the work of Dürck[8] who, by means of intratracheal insufflation was unable to infect the lungs of rabbits with freshly isolated cultures of pneumococcus, streptococcus pyogenes, and staphylococcus aureus unless at the same time, or before or after, injurious dust particles, pumice, or "Thomasphosphatmehl," were also blown into the lungs. This sterile dust alone produced pneumonia while sterile street dust did not. He also describes the production of typical pneumonia in rabbits, with secondary invasion of the pneumonic areas by B. coli, sarcinae, or Friedländer's bacillus, by keeping them at 37-41 C. for 16 to 36 hours and then in ice water for 2-7 minutes.

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[6] Handbuch d. path. Mikroorg., Kolle and Wassermann, 1914, vol. 2.

[7] Amer. Rev. Tuberc., 1919, 3, p. 238, and 1920, 3, p. 750.

[8] Deut. Arch. f. klin. Med., 1897, 58, p. 368.

However, these experiments and those made by the method of intrabronchial insufflation, which was introduced by Lamar and Meltzer and used by many others, do not appeal to us as representing what must take place under natural conditions. Bacteria can be inhaled into the deepest parts of the lungs and if they are capable of multiplying there they will produce pneumonia, as in the case of M 5. The fact that virulent pneumococci do not multiply when planted in the lungs of mice by air currents is an interesting fact and deserves further investigation.

SUMMARY AND CONCLUSIONS

When white mice, white rats and guinea-pigs were exposed to finely divided influenza sputum sprays some died of a primary pneumonia, others of a general infection due to a strain of B. enteritidis (type M 5). Since the work of others has shown that these animals may die of spontaneous infection with members of the paratyphoid-enteritidis group we cannot say that these infections were necessarily the sequel to the spray. However, as primary pneumonia could not be produced in mice when M 5 was inoculated through the buccal or gastro-intestinal mucosa, the conjunctiva, subcutis or peritoneal cavity, but only when sprayed, it seems to us likely that something in the sputum sprays produced a change in the pulmonary tissues favoring such secondary localization.

Broth cultures of M 5 contain a soluble toxin which produces marked congestion of the subcutaneous and pulmonary tissues of white mice. This toxin gives rise to an antitoxin when injected into rabbits. Previous inoculation with the toxin did not produce immunity to the development of primary pneumonia by sprayed cultures, nor were we able to immunize against the spray of M 5 cultures by previous subcutaneous inoculations with a dead culture.

The intoxication of mice with the soluble toxin or with killed cultures of M 5 apparently led to infection with M 5 in a small percentage of the used mice. We were not able to find this bacterium in normal mice, nor did spraying mice with virulent pneumococci make it show itself as a secondary invader.

Experiments show that M 5 and virulent pneumococci are inhaled by mice into the deepest alveoli or capillary bronchi of the lungs, and that primary pneumonia follows in the case of M 5 which is capable of growing and producing its toxin there, whereas, the virulent pneumococci gradually disappear.